

WHAT IS CLAIMED IS:

- 2 1. A DNA Polymerase III-type enzyme found in a thermophilic bacterium which  
3 exhibits the following characteristics:  
4 the ability to extend a primer over a long stretch of ssDNA at elevated  
5 temperature;  
6 the ability to be stimulated by a cognate sliding clamp of the type that is  
7 assembled on DNA by a 'clamp' loader (e.g.  $\gamma$  complex);  
8 contains associated clamp loading sub-units that show DNA stimulated  
9 ATPase activity at elevated temperature and/or ionic strength; and  
10 an accessory protein with DNA polymerase-associated 3'-5' exonuclease  
11 activity.
- 1 2. The DNA Polymerase III-type enzyme according to Claim 1 which is isolated  
2 from a thermophilic bacterium selected from *Thermus* and *Thermatoga* species.
- 1 3. The DNA Polymerase III-type enzyme according to Claim 2, wherein the  
2 thermophilic bacterium comprises a member of the *Thermus* species.
- 1 4. The DNA Polymerase III-type enzyme according to Claim 3, wherein the  
2 thermophilic bacterium comprises *Thermus thermophilus*.
- 1 5. The DNA Polymerase III-type enzyme according to Claim 1, which comprises  
2 at least one of the following:  
3 A. a  $\gamma$  subunit having an amino acid sequence selected from the formula  
4 set forth in SEQ ID NOS:4 and 5;  
5 B. a  $\tau$  subunit having an amino acid sequence corresponding to the  
6 formula set forth in SEQ ID NO:2;  
7 C. a  $\epsilon$  subunit having an amino acid sequence corresponding to the  
8 formula set forth in SEQ ID NO:95;

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9 D. a  $\alpha$  subunit comprising an amino acid sequence corresponding to the  
10 formula set forth in SEQ ID NO:87;

11 E. a  $\beta$  subunit having an amino acid sequence corresponding to the  
12 formula set forth in SEQ ID NO:107; and

13 variants, including allelic variants, muteins, analogs and fragments of any of  
14 subparts (A) through (E), and combinations thereof, capable of functioning in DNA  
15 amplification and sequencing.

1 6. The DNA Polymerase III-type enzyme of Claim 1, which includes a  $\gamma$  sub-unit  
2 which exhibits a frameshift as great as -2.

1 7. An isolated polynucleotide encoding a  $\tau$  subunit of a *Thermus thermophilus*  
2 DNA polymerase III-type enzyme, wherein said  $\tau$  subunit has a molecular weight of  
3 about 58,000 daltons as determined by SDS-PAGE under non-reducing conditions.

1 8. An isolated polynucleotide according to Claim 7, wherein said amino acid  
2 residue sequence is represented by the formula shown in SEQ ID NO:2, analogs  
3 thereof, muteins thereof, alleles thereof, and active fragments thereof.

1 9. An isolated polynucleotide according to Claim 7, wherein the  
2 polynucleotide sequence is the polynucleotide sequence of positions 132 to  
3 1713 of SEQ ID NO:1, conserved variants thereof, analogs thereof, active fragments  
4 thereof, and combinations thereof.

1 10. An isolated polynucleotide according to Claim 7, wherein the polynucleotide  
2 is the polynucleotide sequence of positions 1 to 2027 of SEQ ID NO:1, conserved  
3 variants thereof, analogs thereof, active fragments thereof, and combinations thereof.

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- 1 11. An isolated nucleic acid molecule encoding at least a single subunit of a DNA  
2 polymerase III-type enzyme found in a thermophilic bacterium, which nucleic acid  
3 molecule is selected from the group consisting of:
- 4 A. *dnaX*;  
5 B. *dnaQ*;  
6 C. *dnaE*;  
7 D. *dnaN*;  
8 F. variants, including conserved variants, analogs and fragments of any of  
9 subparts (A) through (D), and combinations thereof, capable of functioning in DNA  
10 amplification and sequencing.
- 1 12. The isolated nucleic acid molecule according to Claim 11, wherein said  
2 nucleic acid molecule comprises *dnaX*.
- 1 13. The isolated nucleic acid molecule according to Claim 11, wherein said  
2 nucleic acid molecule comprises *dnaQ*.
- 1 14. An isolated nucleic acid molecule associated with a DNA polymerase III-type  
2 enzyme found in a thermophilic bacterium, wherein said nucleic acid molecule  
3 comprises *dnaA*.
- 1 15. The isolated nucleic acid molecule according to Claim 11, wherein said  
2 nucleic acid molecule comprises *dnaN*.
- 1 16. A subunit of a DNA polymerase III-type enzyme found in a thermophilic  
2 bacterium, which subunit has an amino acid sequence selected from the group  
3 consisting of SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:2; SEQ ID NO:95; SEQ ID  
4 NO:87; SEQ ID NO:107; mutants thereof; alleles thereof; analogs thereof; active  
5 fragments thereof; and combinations thereof.

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- 1 17. The subunit of Claim 16, wherein said subunit has an amino acid sequence  
2 selected from SEQ ID NO:4 and SEQ ID NO:5, and comprises the  $\gamma$  subunit of a  
3 *Thermus thermophilus* DNA polymerase III-type enzyme.
- 1 18. The subunit of Claim 16, wherein said subunit has an amino acid sequence set  
2 forth in SEQ ID NO:2, and comprises the  $\tau$  subunit of a *Thermus thermophilus* DNA  
3 polymerase III-type enzyme.
- 1 19. The subunit of Claim 16, wherein said subunit has an amino acid sequence set  
2 forth in SEQ ID NO:95, and comprises the  $\epsilon$  subunit of a *Thermus thermophilus* DNA  
3 polymerase III-type enzyme.
- 1 20. The subunit of Claim 16, wherein said subunit has an amino acid sequence set  
2 forth in SEQ ID NO:87, and comprises the  $\alpha$  subunit of a *Thermus thermophilus* DNA  
3 polymerase III-type enzyme.
- 1 21. The subunit of Claim 16, wherein said subunit has an amino acid sequence set  
2 forth in SEQ ID NO:107, and comprises the  $\beta$  subunit of a *Thermus thermophilus*  
3 DNA polymerase III-type enzyme.
- 1 22. A vector comprising an isolated nucleic acid molecule taken from Claim 11.
- 1 23. A vector comprising the isolated nucleic acid molecule of Claim 12.
- 1 24. A vector comprising the isolated nucleic acid molecule of Claim 13.
- 1 25. A vector comprising the isolated nucleic acid molecule of Claim 14.
- 1 26. A vector comprising the isolated nucleic acid molecule of Claim 15.

- 1 27. A vector selected from pET*dnaX* and pET*dnaN*.
- 1 28. A host cell comprising at least one of the vectors of Claim 22.
- 1 29. The host cell according to Claim 28, wherein the host cell is a prokaryotic cell.
- 1 30. A host cell comprising a vector according to Claim 23.
- 1 31. A host cell comprising a vector according to Claim 24.
- 1 32. A host cell comprising a vector according to Claim 25.
- 1 33. A host cell comprising a vector according to Claim 26.
- 1 34. The host cell according to Claim 30, wherein the host cell is a  
2 prokaryotic cell.
- 1 35. The host cell according to Claim 31, wherein the host cell is a  
2 prokaryotic cell.
- 1 36. The host cell according to Claim 32, wherein the host cell is a  
2 prokaryotic cell.
- 1 37. The host cell according to Claim 33, wherein the host cell is a  
2 prokaryotic cell.
- 1 38. An isolated DNA which codes for a recombinant DNA polymerase III-type  
2 enzyme, or subunit thereof, from a thermophilic bacterium, consisting essentially of a  
3 DNA fragment which hybridizes in a Southern blot to an isolated DNA fragment  
4 selected from the group consisting of the DNA fragments defined in SEQ ID NO:6

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9           b)       wash: 5 x 20 minutes with wash buffer consisting of 0.5% BSA,  
10   fraction V), 1mM Na<sub>2</sub>EDTA, 40 mM NaHPO<sub>4</sub> (pH 7.2), and 5% SDS.

1 40. A host cell transformed by the vector of Claim 39.

42. A DNA probe which hybridizes to the DNA sequence coding for the  
thermostable DNA polymerase III-type enzyme, or subunit thereof, of Claim 38,  
wherein the DNA probe is selected from the group consisting of SEQ ID NO:6 and  
SEQ ID NO:8.

4 (a) forming a genomic library from the bacterium;  
5 (b) transforming or transfecting an appropriate host cell with the library of step  
6 (a);

(c) contacting DNA from the transformed or transfected host cell with a DNA probe which hybridizes to a DNA fragment selected from the group consisting of the

9 DNA fragments defined in SEQ ID NO:6 and the DNA fragments defined in SEQ ID  
10 NO:8, wherein hybridization is conducted under the following conditions:

11 i) hybridization: 1% crystalline BSA (fraction V) (Sigma), 1 mM EDTA,  
12 0.5 M NaHPO<sub>4</sub> (pH 7.2), 7% SDS at 65°C for 12 hours and;

13 ii) wash: 5 x 20 minutes with wash buffer consisting of 0.5% BSA,  
14 fraction V), 1mM Na<sub>2</sub>EDTA, 40 mM NaHPO<sub>4</sub> (pH 7.2), and 5% SDS;

15 (d) assaying the transformed or transfected cell of step (c) which hybridizes to  
16 the DNA probe for DNA polymerase III-type activity; and

17 (e) isolating a target DNA fragment which codes for the thermostable DNA  
18 polymerase III-type enzyme or subunit thereof.

1 44. An isolated DNA molecule encoding a protein subunit of DNA polymerase  
2 III-type enzyme from a thermophilic bacterium wherein the subunit group is selected  
3 from the group consisting of  $\tau$ ,  $\gamma$  at a -1 frameshift,  $\gamma$  at a -2 frameshift,  $\epsilon$ ,  $\alpha$ , and  $\beta$ .

1 45. The isolated DNA molecule according to Claim 44, wherein the subunit is  $\tau$   
2 and has a molecular weight of 58 kD.

1 46. The isolated DNA molecule according to Claim 45, wherein the protein has an  
2 amino acid sequence corresponding to SEQ ID NO:2.

1 47. The isolated DNA molecule according to Claim 45, wherein the DNA  
2 molecule has a nucleotide sequence corresponding to SEQ ID NO:3.

1 48. The isolated DNA molecule according to Claim 44, wherein the subunit is  $\gamma$  at  
2 a -1 frameshift, and has a molecular weight of 50.8 kD.

1 49. The isolated DNA molecule according to Claim 46, wherein the protein has an  
2 amino acid sequence corresponding to SEQ ID NO:4.

- 1 50. The isolated DNA molecule according to Claim 44, wherein the subunit is  $\gamma$  at  
2 a -2 frameshift, and has a molecular weight of 49.8 kD.
- 1 51. The isolated DNA molecule according to Claim 47, wherein the protein has an  
2 amino acid sequence corresponding to SEQ ID NO:5.
- 1 52. An expression system comprising an isolated nucleic acid molecule according  
2 to Claim 11.
- 1 53. The expression system according to Claim 52, wherein the protein corresponds  
2 to  $\tau$  and has an amino acid sequence corresponding to SEQ ID NO:2.
- 1 54. The expression system according to Claim 53, wherein the DNA molecule has  
2 a nucleotide sequence corresponding to SEQ ID NO:3.
- 1 55. The expression system according to Claim 52, wherein the protein corresponds  
2 to the  $\epsilon$  subunit and has an amino acid sequence corresponding to SEQ ID NO:95.
- 1 56. The expression system according to Claim 55, wherein said subunit has a  
2 nucleotide sequence corresponding to SEQ ID NO:94.
- 1 57. The expression system according to Claim 52, wherein the protein corresponds  
2 to the  $\alpha$  subunit and has an amino acid sequence corresponding to SEQ ID NO:87.
- 1 58. The expression system according to Claim 57, wherein said subunit has a  
2 nucleotide sequence corresponding to SEQ ID NO:86.
- 1 59. The expression system according to Claim 52, wherein the protein corresponds  
2 to the  $\beta$  subunit and has an amino acid sequence corresponding to SEQ ID NO:107.

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- 1 60. The expression system according to Claim 59, wherein said subunit has a  
2 nucleotide sequence corresponding to SEQ ID NO:106.
- 1 61. A host cell transformed with a heterologous nucleic acid molecule according  
2 to Claim 11.
- 1 62. The host cell according to Claim 61, wherein the protein has an amino acid  
2 sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID  
3 NO:5 SEQ ID NO:87, SEQ ID NO:95, and SEQ ID NO:107.
- 1 63. The host cell according to Claim 61, wherein the nucleic acid molecule has a  
2 nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID  
3 NO:3, SEQ ID NO:86, SEQ ID NO:94, and SEQ ID NO:106.
- 1 64. A DNA probe which hybridizes to the DNA sequence coding for the  
2 thermostable DNA polymerase III-type enzyme of Claim 1, wherein the DNA probe is  
3 selected from the group consisting of the oligonucleotide defined in SEQ ID NO:6;  
4 the oligonucleotide defined in SEQ ID NO:8; the oligonucleotide defined in SEQ ID  
5 NO:10; the oligonucleotide defined in SEQ ID NO:11; the oligonucleotide defined in  
6 SEQ ID NO:12; the oligonucleotide defined in SEQ ID NO:13; the oligonucleotide  
7 defined in SEQ ID NO:14; the oligonucleotide defined in SEQ ID NO:15, and the  
8 oligonucleotide defined in SEQ ID NO:16.
- 1 65. A method for isolating a target DNA fragment consisting essentially of  
2 a DNA coding for a thermostable DNA polymerase III-type enzyme, or subunit  
3 thereof, from a thermophilic bacterium comprising the steps of:  
4 (a) forming a genomic library from the bacterium;  
5 (b) transforming or transfecting an appropriate host cell with the library of step  
6 (a);

7 (c) contacting DNA from the transformed or transfected host cell with a DNA  
8 probe of Claim 61, wherein hybridization is conducted under the following  
9 conditions:

10 i) hybridization: 1% crystalline BSA (fraction V) (Sigma), 1 mM EDTA,  
11 0.5 M NaHPO<sub>4</sub> (pH 7.2), 7% SDS at 65°C for 12 hours and;

12 ii) wash: 5 x 20 minutes with wash buffer consisting of 0.5% BSA,  
13 fraction V), 1mM Na<sub>2</sub>EDTA, 40 mM NaHPO<sub>4</sub> (pH 7.2), and 5% SDS;

14 (d) assaying the transformed or transfected cell of step (c) which hybridizes to  
15 the DNA probe for DNA polymerase activity; and

16 (e) isolating a target DNA fragment which codes for the thermostable DNA  
17 polymerase III-type enzyme or subunit thereof.

1 66. A method for amplifying a nucleic acid molecule, said method comprising  
2 contacting said nucleic acid molecule with a composition comprising the DNA  
3 polymerase III-type enzyme, or subunit thereof, of Claim 1.

1 67. A DNA molecule amplified by the method of Claim 66.

1 68. A method of preparing a recombinant vector comprising inserting a nucleic  
2 acid molecule taken from Claim 11 into a vector.

1 69. The method of Claim 68, wherein said vector is an expression vector.

1 70. A recombinant vector made according to the method of Claim 68.

1 71. A method of making a recombinant host cell, comprising inserting the nucleic  
2 acid molecule of Claim 11 into a host cell.

1 72. The method of Claim 71, wherein said host cell is a bacterial cell, a yeast cell  
2 or an animal cell.

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- 1 73. A kit for amplifying a nucleic acid molecule comprising a carrier and at least
- 2 two containers, wherein at least the DNA polymerase III-type enzyme of Claim 1 is
- 3 disposed in a first container, and the second container holds other reagent necessary or
- 4 useful for amplifying said nucleic acid molecule.

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